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Original Article

Cohorting KPC+ *Klebsiella pneumoniae* (KPC-Kp)–positive patients: A genomic exposé of cross-colonization hazards in a long-term acute-care hospital (LTACH)

Shawn E. Hawken PhD[1], Mary K. Hayden MD[3], Karen Lolans BS[3], Rachel D. Yelin MPH[3], Robert A. Weinstein MD[3], Michael Y. Lin MD[3] and Evan S. Snitkin PhD[1,2]

[1]Department of Microbiology & Immunology, University of Michigan Medical School, Ann Arbor, Michigan, [2]Division of Infectious Diseases, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan and [3]Division of Infectious Diseases, Rush University Medical Center, Chicago, Illinois

Abstract

Objective: Cohorting patients who are colonized or infected with multidrug-resistant organisms (MDROs) protects uncolonized patients from acquiring MDROs in healthcare settings. The potential for cross transmission within the cohort and the possibility of colonized patients acquiring secondary isolates with additional antibiotic resistance traits is often neglected. We searched for evidence of cross transmission of KPC+ *Klebsiella pneumoniae* (KPC-Kp) colonization among cohorted patients in a long-term acute-care hospital (LTACH), and we evaluated the impact of secondary acquisitions on resistance potential.

Design: Genomic epidemiological investigation.

Setting: A high-prevalence LTACH during a bundled intervention that included cohorting KPC-Kp–positive patients.

Methods: Whole-genome sequencing (WGS) and location data were analyzed to identify potential cases of cross transmission between cohorted patients.

Results: Secondary KPC-Kp isolates from 19 of 28 admission-positive patients were more closely related to another patient's isolate than to their own admission isolate. Of these 19 cases, 14 showed strong genomic evidence for cross transmission (<10 single nucleotide variants or SNVs), and most of these patients occupied shared cohort floors (12 patients) or rooms (4 patients) at the same time. Of the 14 patients with strong genomic evidence of acquisition, 12 acquired antibiotic resistance genes not found in their primary isolates.

Conclusions: Acquisition of secondary KPC-Kp isolates carrying distinct antibiotic resistance genes was detected in nearly half of cohorted patients. These results highlight the importance of healthcare provider adherence to infection prevention protocols within cohort locations, and they indicate the need for future studies to assess whether multiple-strain acquisition increases risk of adverse patient outcomes.

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Cohorting of patients who are colonized or infected with high-priority healthcare pathogens has been demonstrated to prevent the spread of healthcare-associated infections (HAIs).[1] Cohorting works by physically separating colonized or infected patients together in an area for care, thereby preventing contact with other patients.[1] In addition to being effective in outbreak settings,[2-4] cohorting reduces cross transmission in endemic healthcare settings with high colonization pressure, such as long-term acute-care hospitals (LTACHs).[5,6]

Carbapenem-resistant Enterobacteriaceae (CRE) are multidrug-resistant organisms (MDROs) that are resistant to nearly all antibiotics and that are estimated to be responsible for 8,500 infections and 1,100 deaths in the United States annually.[7] CRE have been labeled an urgent public health threat for nearly a decade, but despite widespread attention, infections with CRE have not decreased.[7] Previous work has shown that LTACHs have a disproportionately high prevalence of CRE and that they likely contribute to transmission across regions.[8,9] Encouragingly, a recent study demonstrated the effectiveness of a bundled intervention that included cohorting CRE-positive patients to reduce a particular type of CRE *Klebsiella pneumoniae* that carry the KPC-type of carbapenemase (KPC-Kp) in an LTACH with high KPC-Kp prevalence.[10] This study highlights the potential for infection prevention interventions to reduce transmission in these complex and healthcare settings with a heavy burden of MDROs.[10]

Current (2019) guidelines from the CDC for preventing transmission in healthcare settings recommend placing "together in the same room (cohort) patients who are infected or colonized with the same pathogen" when single-patient rooms are unavailable.[1] Yet molecular and phenotypic analyses of prominent healthcare pathogens like CRE indicate that strains of a given antibiotic

Author for correspondence: Evan Snitkin, E-mail: esnitkin@umich.edu.

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resistance type are not necessarily equivalent in terms of resistance mechanisms and virulence genes.[11,12] Cross transmission of genetically diverse strains among cohorted patients could have clinically important consequences. First, patients are often treated empirically based on susceptibility results from prior cultures.[13-15] However, if a patient acquires a new strain, this empiric antibiotic treatment strategy may fail because the secondary organism could carry different antibiotic resistance genes and therefore have a different susceptibility profile.[12,16,17] Additionally, recent reports provide evidence in support of horizontal transfer of antibiotic resistance genes within patients,[18,19] indicating that cocolonization with multiple strains can lead to entry of resistance genes into new genetic backgrounds.

Here, we examined the potential for multiple-strain colonization with KPC-Kp in a convenience sample of patients from a comprehensive surveillance study of KPC-Kp colonization in a Chicago LTACH.[10] We hypothesized that by integrating whole-genome sequencing (WGS) and patient location data we would identify KPC-Kp–colonized patients with evidence of acquisition of distinct secondary KPC-Kp strains through cross transmission from other patients cohoused in cohort locations. Moreover, we predicted that subsequently acquired strains would harbor antibiotic resistance genes that were not found in the patient's admission isolate.

Methods

LTACH setting, study design, and sample collection

Detailed information regarding the study design, intervention bundle, and data collection are available in the study by Hayden et al.[10] Briefly and of relevance to the current manuscript, the 1-year study took place between 2011 and 2013 during a quality improvement project to prevent KPC-Kp colonization and infection in a Chicago LTACH where the average census was 99 patients and the prevalence of KPC-Kp colonization was 30%. All location data and isolates presented here were collected at a single LTACH (LTACH C) during the intervention period, which included surveillance swab culture–based direct ertapenem disk screening of all LTACH patients for KPC-Kp rectal colonization at LTACH admission and every 2 weeks thereafter (94% adherence), as well as increased hand hygiene at room exit (69% adherence), donning gown and gloves when caring for patients in high-acuity rooms (86% adherence), and efforts to separate KPC-Kp–positive and KPC-Kp–negative patients by placing KPC-Kp–positive patients in ward cohorts (91% adherence).[6,20] All patient rooms were single or double occupancy.

Longitudinal convenience sample of KPC-Kp isolates from previously colonized patients

During the original study, the first KPC-Kp surveillance isolate was collected from each colonized patient.[10] Once a patient was found to be colonized with KPC-Kp, the patient was presumed to remain colonized indefinitely. Colonized patients were not rescreened systematically; however, additional 'secondary' KPC-Kp isolates were collected from a subset of patients whose prior colonization status was unclear to study staff at the time of screening.

The current analyses are restricted to this longitudinal, convenience sample of patients who were KPC-Kp positive at the study start (imported KPC-Kp) or upon LTACH admission (within 3 days) and who also had 1 or more additional KPC-Kp surveillance isolates collected later. These admission-positive patients were selected for study because they were housed in cohort locations during their entire LTACH stay, providing long periods of exposure to other KPC-Kp–positive patients and potential opportunities for cross transmission.

Sample preparation and whole-genome sequencing

Frozen glycerol stocks of surveillance isolates were stored at −80°C. Frozen stocks were streaked onto Luria-Bertani agar, and unique morphological growth was collected for DNA preparation. DNA was extracted with the PowerMag Microbial DNA kit (Mo Bio, Carlsbad, CA) and prepared for sequencing on an MiSeq instrument (Illumina, San Diego, CA) using the NEBNext Ultra kit (Illumina) and sample-specific barcoding. Library preparation and sequencing were performed at the Center for Microbial Systems at the University of Michigan or the University of Michigan Sequencing Core. The quality of reads was assessed with FastQC software,[21] and we used Trimmomatic software[22] to trim adapter sequences and low-quality bases. Assemblies were performed using the A5 pipeline with default parameters.[23] Sequence data are available under BioProject (no. PRJNA603790).

Identification of single nucleotide variants

Single nucleotide variation (SNV) calling was performed as described in Han et al[24] using a variant calling pipeline (https://github.com/Snitkin-Lab-Umich/variant_calling_pipeline). To summarize, variant calling was performed with SAMtools[25] using closed-genome assembly multilocus sequence type (MLST)–specific reference genomes listed in Supplementary Table 1 (online).

Assessment of epidemiologically supported secondary acquisitions linked to other LTACH patients and roommates

Epidemiologically plausible donor patient isolates were defined as isolates collected before the recipient patient's secondary isolate collection date. To account for acquisition potentially occurring between surveillance sampling dates, the positive donor time frame for all analyses was defined starting on the date of the donor's last negative swab before the collection date of the putative donor isolate.

Shared spatiotemporal exposures between patients, which plausibly facilitated secondary acquisition between roommates, were assessed. Plausible cross transmission events leading to acquisition of secondary strains were defined as isolation of a strain in a recipient patient previously negative for this strain and the recipient sharing time and space with a donor patient who was positive for this strain prior to the recipient.

Genetic relationships between KPC-Kp isolates based on SNV distance

Pairwise distances were calculated from core and accessory genome single-nucleotide variants (SNVs) in detected in MLST-specific WGS alignments (Supplementary Table 1 online). SNV distances were compared (1) between the first (primary) isolate and subsequently collected (secondary) isolates from the same admission-positive patient and (2) between secondary isolates from admission-positive patients and isolates from other plausible donor patients in the LTACH.

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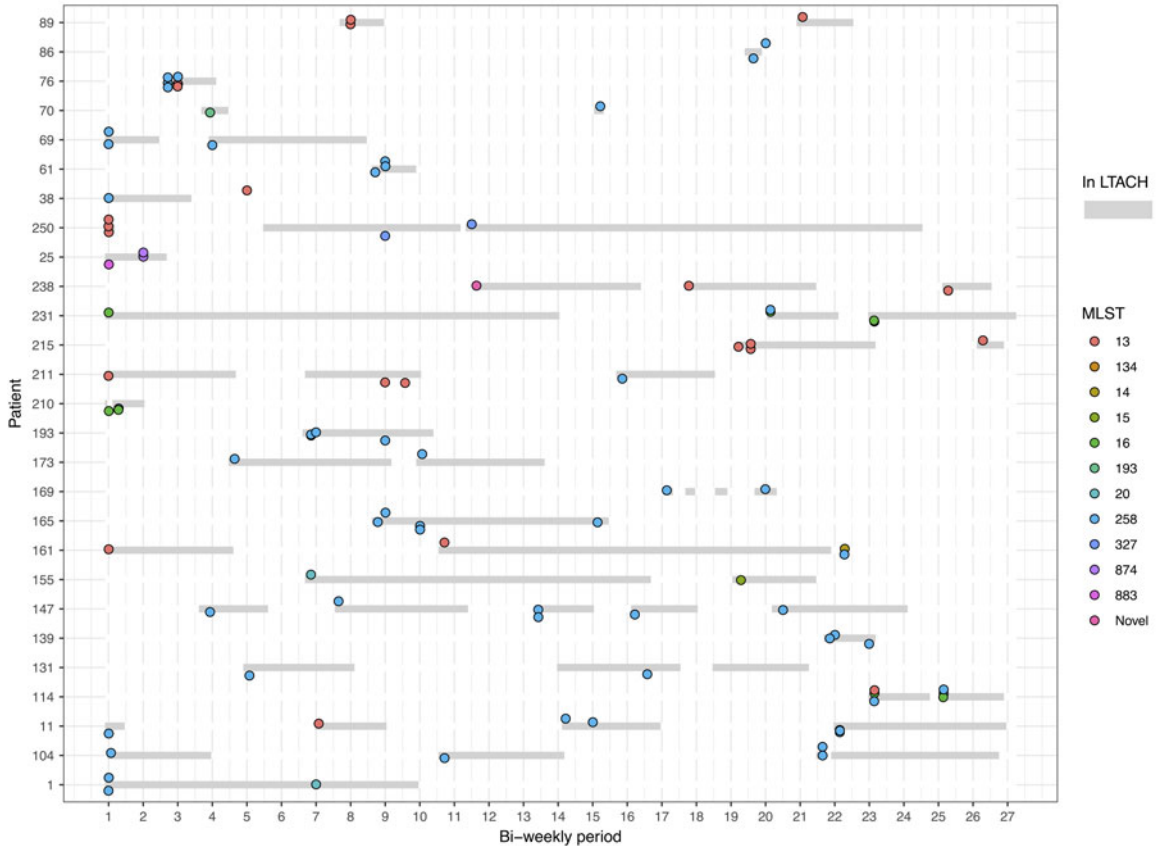

Fig. 1. KPC-Kp isolates from convenience sample of patients who were positive at the study start or admission to the long-term acute-care hospital (LTACH). Patients (N = 28) have primary and secondary isolates that are from the same multilocus sequence type (MLST), a different MLST, or both same and different MLSTs. *Y*-axis indicates patients; *X*-axis indicates biweekly time periods during the study; circles indicate positive culture dates and are colored by the MLST of the isolate collected. Grey bars indicate when patients were in the LTACH.

Detection of resistance genes in whole-genome sequences

Kleborate software (https://github.com/katholt/Kleborate) was used to screen whole-genome sequence (WGS) assemblies for presence of genes and mutations known to confer antibiotic resistance in *K. pneumoniae*. We used a custom R script to expand antibiotic resistance gene alleles reported from Kleborate into gene presence absence profiles (Supplementary Table 1 online), counting only the Kleborate-reported precise matching gene hits as being present or absent.

Results

Almost half of cohorted patients acquired secondary isolates of a new sequence type

Among the admission-positive patients who had secondary isolates available, 100% were cohorted per protocol: 21 patients with 46 secondary isolates shared a room with at least 1 patient who was KPC-Kp–positive before their secondary isolate being collected, and 8 patients with 15 secondary isolates did not have overlap with a positive patient before their secondary isolate was collected, but were instead housed in single patient rooms during the acquisition time frame for these isolates. Isolates from the 21 patients who shared a room with a putative KPC-Kp–positive donor prior to secondary acquisition were collected after patients shared a room with positive patients for a median of 51 days (range, 1–132 days) prior to detection of a secondary isolate.

We considered 127 'admission-positive' patients, who were either positive at the start of the study or on first admission to the LTACH, for potential acquisition of secondary KPC-Kp strains during their stay. Although the original sampling strategy was not designed to track longitudinal colonization of KPC-Kp,[10] 28 admission-positive patients, in addition to their 38 primary (earliest) isolates collected on admission or study start, also had 63 secondary (subsequent) isolates collected later in their LTACH stays (Fig. 1). Of the 101 isolates available from these admission-positive patients, we extracted quality WGS data from 99 isolates including 38 primary and 61 secondary isolates. Although most primary and secondary isolates were from the epidemic ST258 strain (55% of primary isolates, 57% of secondary isolates), a diversity of other MLSTs was observed among both primary and secondary isolates (Table 1). Secondary isolates were collected from patients a median of 89 days (range, 1–310 days) after primary isolates. Evaluation of MLSTs of the primary and secondary KPC-Kp isolates provided support for secondary acquisition among cohorted patients, with 13 (46%) patients having a distinct secondary MLST that was not detected at admission.

Genomic evidence of potential secondary acquisitions from other LTACH patients among admission-positive patients

To assess genomic evidence of cross transmission in the cohort, we evaluated the fraction of patients whose secondary isolates were more closely related to another patient's isolate than to their

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Table 1. Frequency of Strong Genetic Relationships Between Secondary Isolates and Isolates from other patients[a]

SNV Distance Between Secondary Isolates and Isolates From Another Patient	<25 SNV	<10 SNV	<5 SNV
Isolate from another LTACH patient	17 patients	14 patients	11 patients
	26 isolates	21 isolates	12 isolates
Isolate from patient on cohort floor	15 patients	12 patients	10 patients
	19 isolates	15 isolates	11 isolates
Isolate from roommate in cohort	5 patients	4 patients	3 patients
	6 isolates	5 isolates	3 isolates
Time between primary and secondary isolate detection, median days (range)	153 (86–298)	133 (86–298)	99 (86–298)
Duration of exposure to roommate in cohort, median days (range)	12[b] (2–49)	12 (2–49)	13 (2–49)

Note. SNV, single nucleotide variant; LTACH, long-term acute-care hospital.
[a]From other patients among patients whose primary isolate is most closely related to another patient's isolate. Total admission-positive patients with secondary isolates: 28. Total secondary isolates: 63.
[b]Indicates the sum of the duration of exposure to multiple plausible patient donors in the <25 SNV range per recipient patient.

own primary isolate (Fig. 2). Of the 28 admission-positive patients with 1 or more secondary isolates, 19 had a secondary isolate that was more closely related to another patient's isolate than to their own primary isolate. Of those 19 patients, 17 had secondary isolates that were more closely related to an isolate from a patient with whom they overlapped on the cohort floor and 8 had secondary isolates that were more closely related to an isolate from a roommate. Plausible transmission in the cohort was further supported by extremely small SNV distances in most of these cases, with 12 patients' isolates being within 10 SNVs of another patient's isolate on the cohort floor and 4 patients' isolates being within 10 SNVs of an isolate from a roommate (Table 1).

Patients accumulate diverse antibiotic resistance genes in association with acquisition of a secondary KPC-Kp isolate

An abundance of molecular and genomic evidence indicates that members of the same bacterial species, including KPC-Kp, can vary extensively in the arsenal of antibiotic resistance genes encoded in their chromosomes and plasmids.[11,26,27] To determine whether secondary acquisitions resulted in increased antibiotic resistance potential, we examined whether patients with high-confidence putative transmission links (<10 SNVs to another patient's isolate and >10 SNVs from their own primary isolate) acquired additional unique resistance genes in their secondary isolate. Compared with a patient's primary isolate, secondary isolates contributed a median of 2.5 additional antibiotic resistance genes beyond the primary isolate (minimum 0, maximum 10 additional resistance genes) (Table 2). In total, additional resistance genes were gained in 12 of the 14 patients whose secondary isolates had strong genomic links to isolates from other patients, including 3 patients whose secondary isolates were linked to patients with whom they had shared a cohort room prior to secondary isolate

Table 2. Summary of Antibiotic Resistance Genes Among Primary, Secondary, and All Isolates From Admission-Positive Patients Whose Secondary Isolate Was Most Closely Related to and Within 10 SNVs of Another Patient's Isolate

Gene	Minimum	Median	Maximum
Antibiotic resistance genes detected in primary isolates	4	9.5	13
Antibiotic resistance genes detected in secondary isolates	0	2.5	10
Total unique antibiotic resistance genes in primary and secondary isolates	4	13	18

Note. SNV, single nucleotide variant.

acquisition (Fig. 3 and Supplementary Table 1 online). Patients with unlinked secondary isolates accumulated fewer additional resistance genes (median 0, minimum 0, maximum 2 additional resistance genes) (Supplementary Fig. 1 online). This finding supports the hypothesis that these closely related isolates (<10 SNVs) represented primary isolates that accrued mutations over the course of prolonged colonization rather than the hypothesis that patients acquired a secondary KPC-Kp strain via transmission from another patient.

Discussion

Cohorting patients who are colonized or infected with MDROs is an effective strategy to reduce the risk of MDRO transmission to uncolonized patients. However, little attention has been given to the potential for cohorted patients themselves to acquire secondary resistant strains through exposure to the high colonization pressure of MDROs within cohorts. Secondary strain acquisition may be particularly important in endemic settings where the MDRO for which patients are cohorted (eg, CRE), may comprise a heterogeneous group of bacteria with varying genetic potential. To investigate this risk, we performed a genomic epidemiologic investigation of a longitudinal, convenience sample of KPC-Kp isolates from patients on cohort floors in an LTACH. We found strong evidence of cross transmission within cohorts, with secondary isolates often harboring antibiotic resistance genes not found within a patient's primary isolate.

Our finding that secondary isolates carry antibiotic resistance potential that is distinct from that found in patients' primary isolates is noteworthy because it suggests that multiple-strain acquisition could increase the risk of treatment failure. Acquisition of a secondary strain that is resistant to antibiotics to which the primary strain was susceptible could be particularly problematic for highly resistant organisms like KPC-Kp, for which treatment options are already limited. For example, colistin/polymyxin E is a last-resort drug used to treat severe multidrug-resistant gram-negative infections, such as those due to KPC-Kp.[28-31] In our study, a single patient plausibly acquired a secondary isolate with predicted colistin resistance that was linked within 25 SNVs of another LTACH patient's isolate (Supplementary Table 1 online). Because colonization is a major risk factor for KPC-Kp infection[32-34] and because infections are thought to arise primarily from the patient's colonizing strain,[35] the acquisition of a colistin-resistant isolate could limit efficacious treatment options and in turn increase mortality risk.[31,36] In addition to the potential risks to multiply colonized patients, the acquisition of strains with different resistance arsenals provides an opportunity for horizontal gene exchange

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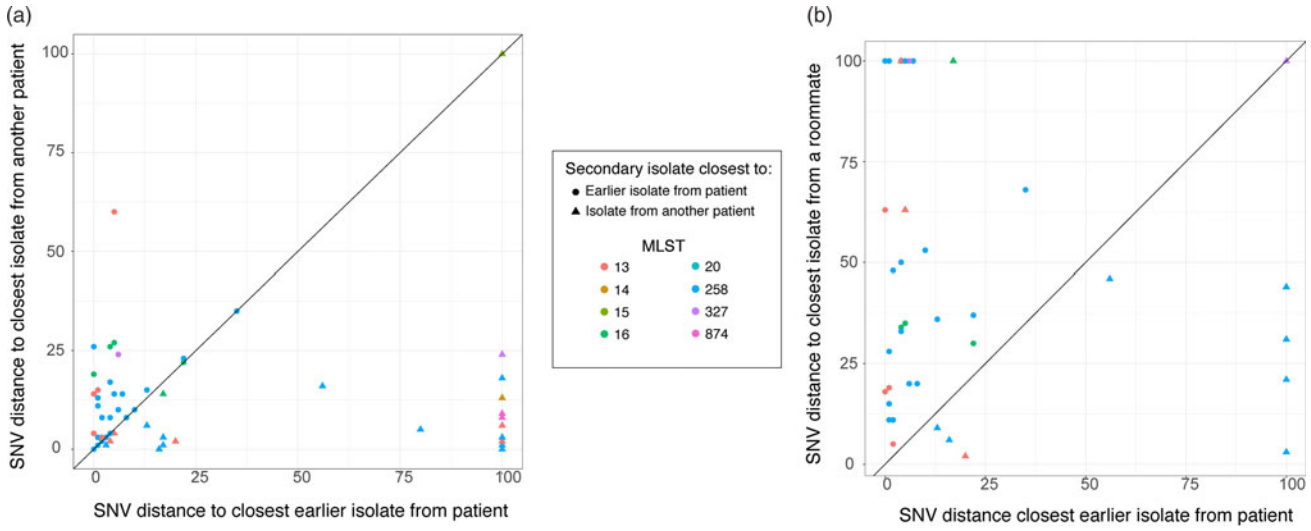

Fig. 2. Genetic relationship between a patient's primary and secondary isolates compared to isolates from other patients in the long-term acute-care hospital (LTACH) and room cohorts. Pairwise single-nucleotide variance (SNV) distance between secondary isolates and closest primary isolate from the same patient compared to closest related isolate from (A) another patient in the facility or (B) a cohorted roommate. Diagonal line separates secondary isolates that are more closely related to primary isolates from the same patient (above the diagonal) or to another patient's isolate (below the diagonal). Colors indicate the MLST multilocus sequence type (MLST) of the secondary isolate. Circles indicate the closest genetic relative to the isolate by SNV distance is from the same patient (eg, the patient's own primary isolate), and triangles indicate that the closest relative was isolated from another patient. Comparison of isolates from different MLSTs or >100 SNVs are collapsed into the >100 SNV category for plot visualization purposes.

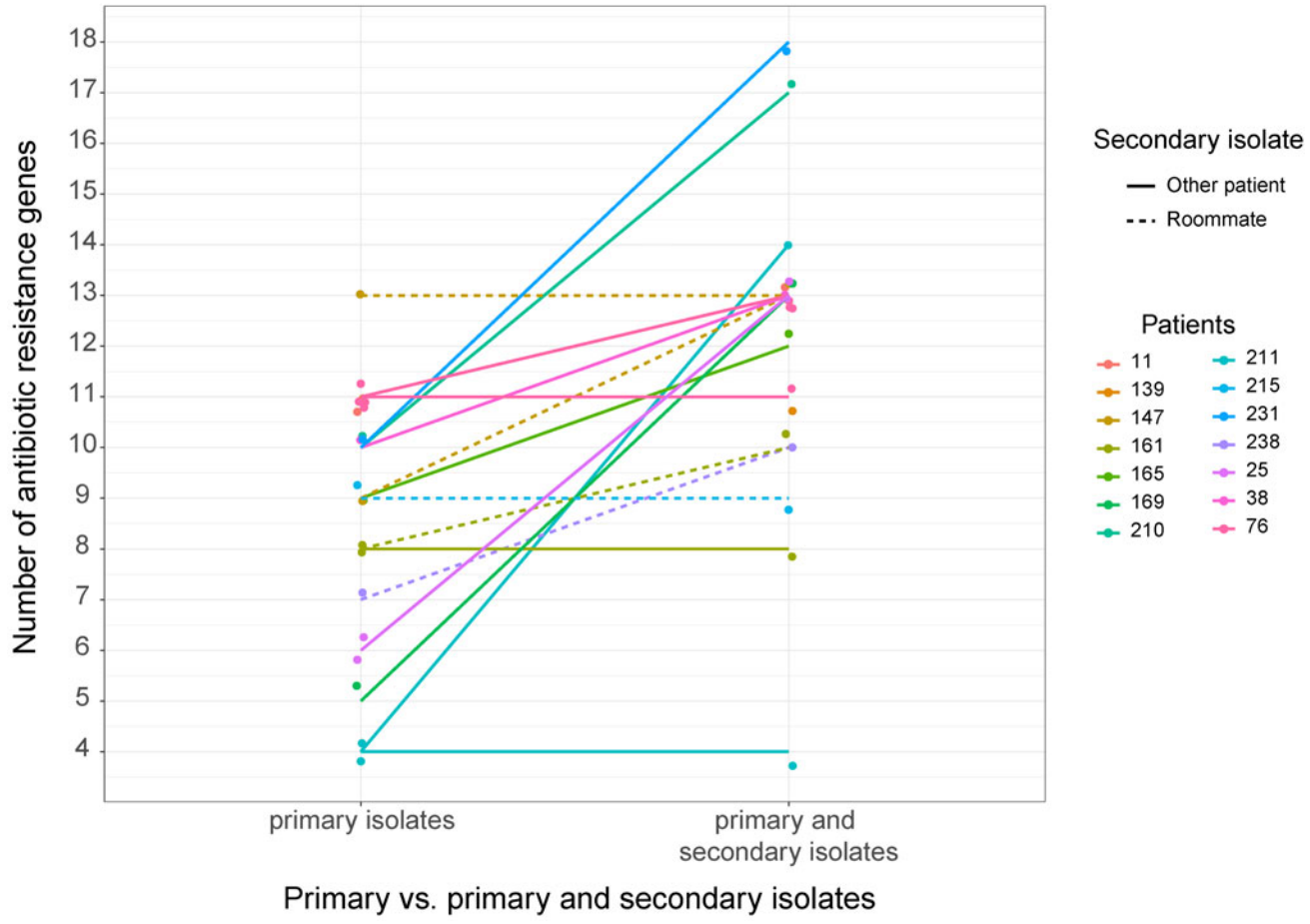

Fig. 3. Number of antibiotic resistance genes detected in genomes from primary isolates compared to primary and secondary isolates from admission-positive patients whose secondary isolates are linked with high confidence (<10 single-nucleotide variances or SNVs) to isolates from other patients in the long-term acute-care hospital (LTACH). *Y*-axis indicates number of unique resistance genes detected with Kleborate (see Methods, and Supplementary Table 1 online), *X*-axis indicates number of unique antibiotic resistance genes detected among primary (left) and primary and secondary isolates (right). Colors distinguish patients. Dashed lines indicate patients whose secondary isolate is within 10 SNVs of an isolate from a cohorted roommate.

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and the accumulation of resistance within a single transmissible strain.[18,19,37] Moreover, harboring genetically diverse strains creates an opportunity for resistance alleles to find their way to strains with other clinically relevant characteristics, such as hypervirulence[12,38-40] or epidemic potential.[39] Additional risk to patients could stem from the fact that different strains of the same pathogen often carry different virulence genes.[37] Virulence factor differences in acquired strains may predispose patients to developing infections of different types and severity.[37,38]

In addition to potentially making infections more difficult to treat, acquisition of secondary strains could also increase a patient's time at risk of infection by prolonging the total period of colonization. All of these potential adverse consequences of multiple-strain colonization emphasize the importance of protecting previously colonized patients from secondary acquisition and for healthcare providers to adhere to infection prevention protocols, even when caring for patients in cohort locations.

Our study has several limitations. First, we studied a convenience sample, which inherently precludes systematic calculation of risk. Second, we conducted limited sequencing of multiple clones from the same sample—a single representative of unique morphologies observed in each sample, primarily a single clone per sample. This method hindered our ability to determine whether a patient was simultaneously colonized with multiple strains (eg, colonized by both their primary and secondary strains at the same time). These sampling limitations also prevented us from determining whether patients remained colonized with their primary strain when they became colonized with their secondary strain, or if colonization with both strains persisted. Thus, it is possible that cohort patients entered the facility already colonized with multiple strains or acquired colonization with secondary strains inside or outside the LTACH prior to being moved to cohort locations and did not acquire their secondary strains in the cohort. Although we cannot definitively rule out this possibility, the acquisition of secondary strains in the LTACH is supported by the finding that 14 of the 28 patients with secondary isolates had strong genomic links (<10 SNVs) to other LTACH patients including cohorted roommates (Supplementary Fig. 2 online). In total, these 14 strong genomic linkages accounted for 50% of the 28 admission-positive patients with multiple isolates available and 11% of the 127 admission-positive patients in the full study.

In summary, our study provides strong evidence for cross transmission of KPC-Kp strains within a KPC-Kp–positive cohort, with accumulation of new antibiotic resistance genes by patients who acquired secondary KPC-Kp strains. Whether acquisition of multiple KPC-Kp strains increases risk of adverse patient outcomes needs to be studied further. In the meantime, we recommend robust adherence to infection prevention precautions within KPC-Kp cohorts to reduce the risk of within-cohort cross transmission of KPC-Kp strains.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2020.261

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Conflicts of interest. All authors report no conflicts of interest relevant to this article.

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